Bulgarian Chemical Communications, Volume 52, Issue A (pp. 53-58) 2020 DOI: 10.34049/bcc.52.A.185

The trace element contents in wild edible mushrooms samples and econometric modeling of data

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Received August 11, 2019; Accepted December 15, 2019

The aims of this work were to determine trace elements (Pb, Cd, Co, Cu, Mn, Zn and Fe) contents in the wild edible mushroom *Suillus luteus* growing in the Batak Mountain, Bulgaria and to identify the relationship between Mn and the other elements using ordinary least squares multiple linear regression models. Quantitative determination of the concentration of the studied trace elements was carried out in mineralized samples on a Perkin Elmer AAnalyst 800 atomic absorption spectrometer with deuterium background corrector. All statistical computing, analysis and all charts were performed with the statistical software R program. We obtained an ordinary least squares multiple linear regression model showing that: if Fe and Cd increase by 1%, the effect of this increase would result in an increase in Mn by 8.17% on the average; if Zn, Cu, Co and Pb decrease by 1%, the effect of this decrease would result in a decrease in Mn by 1.21% on the average.

Keywords: Atomic absorption spectrometry, Trace elements, Linear regression model, Ordinary least squares, *Suillus luteus*

INTRODUCTION

According to the Food and Agriculture Organization of the United Nations, 1350 species are considered as edible mushrooms. There is no doubt that mushrooms play an important role in human dietary, especially in regions with deep and old tradition of mushrooming [1, 2]. In many countries, picking wild grown mushrooms is a habitual activity for example in 2014 in Poland reported 5212 tons of forest-growing mushrooms purchased from pickers, worth more than 16 million euro [3]. China is the leading producer, user and exporter of wild edible mushrooms fin the world (including wild-growing and cultivated mushrooms) [4]. According to FAO, Bulgaria is Europe's leading exporter of mushrooms and major sources of revenue [4, 5]. The edible mushrooms are an increasing fraction of human diet for example, in Bulgaria, each person consumes over 6 kg of fresh mushrooms per year.

Wild-growing saprobic and symbiotic mushrooms may accumulate in their fruiting bodies' considerable amounts of metallic elements and metalloids due to specificities in their physiology [6-9]. The known essential micronutrient minerals are iron, zinc, selenium, manganese, cobalt and copper. These microminerals play an important role in the catalytic processes within the enzyme system that includes a wide range of enzyme activities associated with metabolic, endocrine and immune systems [10-12]. Living organisms require trace of some trace elements, including iron, cobalt, copper, manganese, chromium and zinc. Excessive levels of these elements, however, can be detrimental to human health. Lead and cadmium are non-essential elements as they are toxic, although their trace amounts are not known for any beneficial effects on organisms [13-15]. Furthermore, deficiency or imbalance of essential or non-essential minerals above threshold concentration levels can put the individual at risk of disease development. Therefore, the adequate and accurate food composition data are inevitable for estimating the adequacy of intakes of essential nutrients and assessing the risk from toxic elements [16-19].

The aims of this work were to determine trace elements (Pb, Cd, Co, Cu, Mn, Zn and Fe) contents in the wild edible mushroom *Suillus luteus* growing in the Batak Mountain, Bulgaria and to identify the relationship between Mn and the other elements using ordinary least squares multiple linear regression models.

EXPERIMENTAL

Data description

Plant material. Mushroom samples were collected in 2018 from the Batak Mountain, Bulgaria by the authors themselves.

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The Batak Mountain is located in the western Rhodopes. Its western border is defined by the Chepinska river, the southern border - by Dospatska river and Dospat dam, the eastern border - by Vacha river and the northern border - by the Thracian Plane (GPS41°46'02.6"N24°08'48.4"E). The region is industry-free and is characterized with forests, land and low buildings.

Chemical analysis methods. Quantitative determination of the concentration of the studied trace elements (Pb, Cd, Co, Cu, Mn, Zn and Fe) was carried out in the mineralized samples by Perkin Elmer AAnalyst 800 atomic absorption spectrometer with deuterium background corrector.

Digestion procedures. Multiwave 3000 closed vessel microwave system (maximum power was 1400 W, and maximum pressure in the Teflon vessels - 40 bar) was used in this study. Mushroom samples (0.25 g) were digested with 6 mL of HNO_3 (65%) and 1 mL of H_2O_2 (30%) in the microwave digestion system for 23 min and diluted to 25 mL with deionized water. A blank digest was carried out in the same way. All sample solutions were clear.

Statistical analysis

Statistical software. All statistical computing, analysis and all charts were performed with the Statistical software R program version 3.5.1.

Statistical tests. The Shapiro-Wilk test is a test for normal distribution exhibiting high power, leading to good results even with a small number of observations. In contrast to other comparison tests the Shapiro-Wilk test is only applicable to check for normality. The null-hypothesis (H_0): the population is normally distributed. The test statistic is [20]:

$$W = \frac{\left(\sum_{i=2}^{n} a_i y_i\right)^2}{\sum_{i=1}^{n}\left(x_i - \bar{y}\right)^2},$$

where n - number of observations, y_i - values of the ordered sample, a_i - tabulated coefficient.

The Durbin-Watson test statistic tests the null hypothesis that the residuals from an ordinary least squares regression are not autocorrelated. The Durbin-Watson statistic ranges in value from 0 to 4. A value near 2 indicates non-autocorrelation; a value toward 0 indicates positive autocorrelation; a value toward 4 indicates negative autocorrelation. The test statistic is [21]:

$$d = \frac{\sum_{t=2}^{n}\left(e_t - e_{t-1}\right)^2}{\sum_{t=1}^{n} e_t^2},$$

where e_t is the residual associated with the observation at time t and n is the number of observations.

Because of the dependence of any computed Durbin-Watson value on the associated data matrix, exact critical values of the Durbin-Watson statistic are not tabulated for all possible cases. Instead, Durbin and Watson established upper (DU) and lower bounds (DL) for the critical values. Typically, tabulated bounds are used to test the hypothesis of zero autocorrelation against the alternative of positive first-order autocorrelation, since positive autocorrelation is seen much more frequently in practice than negative autocorrelation [22].

The F-test for linear regression tests whether any of the independent variables in a multiple linear regression model are significant. The formula for F-test statistic is:

$$F = \frac{explained\ variance}{unexplained\ variance},$$

F-test is greater as:

- distance between groups is greater or dispersion media groups around the general average are greater;
- groups are more homogeneous or error represented by scattering within the groups is less.

Thus, relatively high F's are strong arguments against H_0 (null hypothesis). P-value is the probability of obtaining a value of F which is at least as great as that observed by us if H_0 were true. Therefore, the smaller the p-value the chances H_0 to be fair are lower. For p-value < 0.05 to reject H_0 it the following condition is necessary:

$$F_{calculat} \text{ f } F_{tabular}$$

$$(Fcalculated > Ftabular)$$

Variance inflation factors (VIF) measure how much the variance of the estimated regression coefficients are inflated as compared to when the predictor variables are not linearly related. If VIF > 10 there is an indication for multicollinearity to be present [23].

Model specification

In econometrics, ordinary least squares (OLS) method is widely used to estimate the parameters of a linear regression model. OLS estimators minimize the sum of the squared errors (a difference between observed values and predicted values). The OLS estimator is consistent when the regressors are exogenous, and optimal in the class of linear unbiased estimators when the errors are homoscedastic and serially uncorrelated. Under these conditions, the method of OLS provides minimum-variance mean-unbiased estimation when the errors have finite variances. Under the additional assumption that the errors are normally distributed, OLS is the maximum likelihood estimator.

In this article we used OLS multiple linear regression model to identify the dependences of trace elements in wild edible mushroom *Suillus luteus*. The long-run function is specified as follows:

Mn = *f* (Fe, Zn, Cu, Co, Cd, Pb).

From the point of view of multiple linear regression the constructed model must satisfy the following assumptions: linear relationship, multivariate normality, no or little multicollinearity, no autocorrelation and homoscedasticity [23].

RESULTS AND DISCUSSION

Econometric modeling

Concentrations of seven trace elements (Pb, Cd, Co, Cu, Mn, Zn and Fe) were determined in this study. The trace element contents of the species depend on the ability of the species to extract elements from the substrate, and on the selective uptake and deposition of elements in tissues [24-29]. The results obtained in the current study indicated that Pb, Cd, Co, Cu, Mn, Zn and Fe contents of the investigated mushroom samples were found to be comparable with those reported in the literature (Table 1) [12, 27, 28, 30-35]. The averages of Pb, Cd, Co, Cu, Mn, Zn and Fe present in the studied wild mushrooms were far below the limits set by the WHO [36]. Descriptive statistics was used to make a preliminary analysis of the database (Table 1).

Table 1. Descriptive statistics of the results for Pb, Cd, Co, Cu, Mn, Zn and Fe concentrations in *Suillus luteus* mushroom (n = 15)

	Pb	Cd	Co	Cu	Mn	Zn	Fe
Mean	7.957	0.973	1.032	6.121	14.457	78.563	84.947
Std. Error	0.379	0.027	0.022	0.486	0.437	1.088	1.818
Median	7.910	0.950	1.028	6.751	14.084	77.901	86.844
Std. Dev.	1.468	0.106	0.083	1.881	1.692	4.215	7.040
Variance	2.155	0.011	0.007	3.537	2.863	17.769	49.566
Kurtosis	-1.148	-0.817	-1.227	-0.580	-1.213	-0.918	-0.341
Skewness	0.015	0.245	-0.389	-0.574	0.034	0.398	-0.778
Range	4.851	0.361	0.237	5.858	5.232	12.057	22.030
Minimum	5.545	0.788	0.889	3.032	11.624	73.156	71.963
Maximum	10.396	1.150	1.126	8.890	16.856	85.213	93.993
Sum	119.357	14.589	15.483	91.819	216.854	1178.452	1274.216
Count	15.000	15.000	15.000	15.000	15.000	15.000	15.000
Conf. Level (95.0%)	0.813	0.059	0.046	1.041	0.937	2.334	3.899
Homogeneous coefficient (%)	18.450	10.905	8.069	30.723	11.705	5.366	8.288
Shapiro-Wilk Test	0.950	0.949	0.892	0.897	0.938	0.912	0.896
p-value	0.532	0.518	0.072	0.085	0.359	0.144	0.0813

Table 2. Econometric modeling for Mn

Coefficient	Estimate	Std. Error	t-Statistic	Pr (> |t|)
a_0	-22.534	12.537	-1.797	0.110
a_1	0.509	0.132	3.862	0.005*
a_2	-0.202	0.116	-1.745	0.119**
a_3	-0.167	0.199	-0.838	0.427
a_4	-4.439	3.845	-1.154	0.282
a_5	15.823	5.809	2.724	0.026*
a_6	-0.018	0.259	-0.068	0.947
Multiple R-squared	0.803	F-statistic		41.446 (p-value = $1.596e^{-04}$)
Adjusted R-squared	0.656	Durbin-Watson stat		1.987 (p-value = 0.07048)
Residual standard error	0.993			

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table 3. Coefficient confidence intervals

Coefficient	Estimate	90%		95%	
		lower	upper	lower	upper
a_0	-22.534	-45.847	0.780	-51.445	6.3778
a_1	0.509	0.264	0.754	0.205	0.812
a_2	-0.202	-0.417	0.013	-0.469	0.065
a_3	-0.167	-0.536	0.203	-0.625	0.292
a_4	-4.439	-11.590	2.711	-13.307	4.428
a_5	15.823	5.021	26.624	2.428	29.218
a_6	-0.018	-0.499	0.464	-0.615	0.579

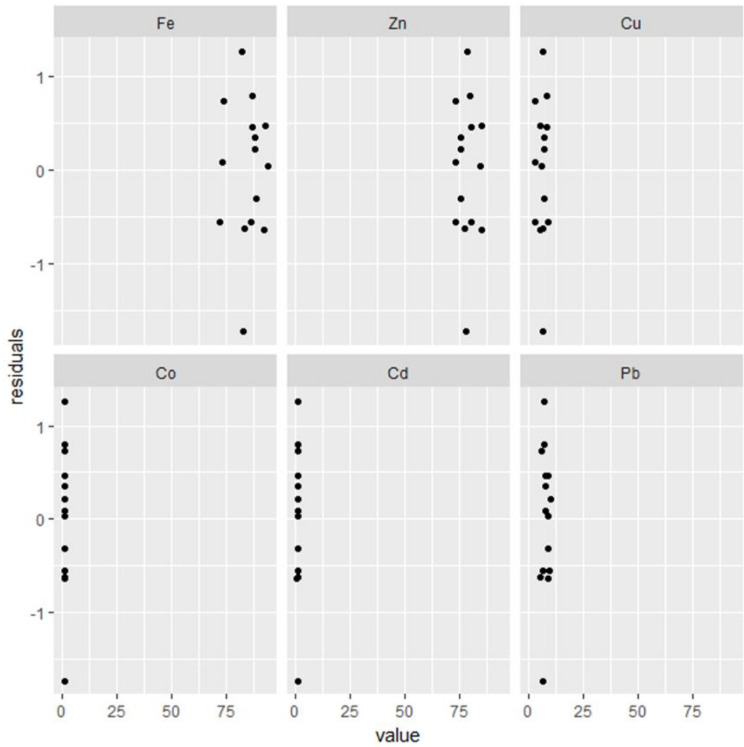

Fig. 1. Plots of the residuals *versus* each regressor

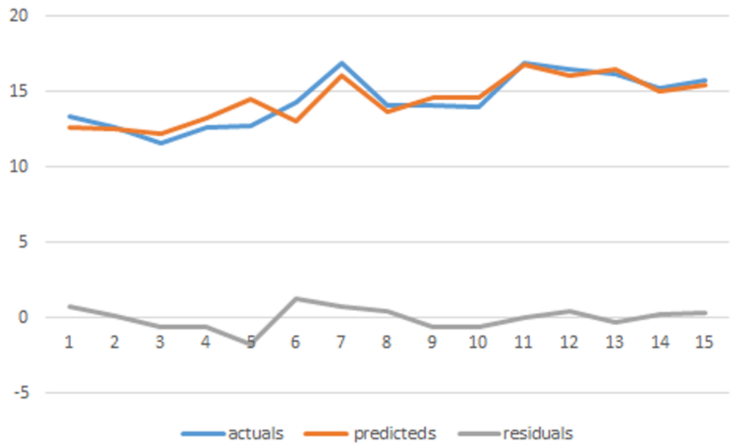

Fig. 2. Differences of actual data *vs*. predicted data from the model for Mn

It was concluded that the data were fairly symmetrical because the skewness was between -0.5 and 0.5, except for Fe and Cu which were negatively skewed (skewness between -1 and -0.5). Data for Co and Fe can be considered homogeneous. From the Shapiro-Wilk Test, the p-value > 0.05 suggested that the distribution of the data was not significantly different from normal distribution. In other words, normality could be assumed.

Econometric model for Mn. In an explicit and econometric form, the long-run function of Mn can be stated as:

$$Mn = a_0 + a_1Fe + a_2Zn + a_3Cu + a_4Co + a_5Cd + a_6Pb$$

For econometric modeling of the variables included in this study factor Mn we considered as a resultant factor and Fe, Zn, Cu, Cu, Co, Cd and Pb: as independent factors (Table 2). At the significance level, three out of seven regression coefficients were statistically significant. The econometric analysis of the model highlighted the fact that the relation between dependent and independent factors was rather strong (Table 2): the correlation coefficient was R = 0.986 and the determination coefficient (R-squared) was 0.803 or roughly 80% of the variance found in the response variable (Mn) can be explained by predictor variables. The Durbin-Watson test indicated non-autocorrelation (test statistic value was near 2, then the null hypothesis was not rejected). The calculated values of the Fisher's F-test (F) of the econometric model indicated the higher relevance of the model (F = 41.446, p-value = $1.596e^{-04}$). Tabular value given by the test was 4.48 for a probability of 0.05 (5%) [37], which means that the resulting equation was: F calculated > F tabular. So the null hypothesis (H_0) was rejected and the variance values included in the study differed significantly between them. The VIF values for the explanatory variables Fe, Zn, Cu, Co, Cd and Pb were: 2.218, 3.385, 1.987, 1.457, 5.393 and 2.055, respectively. Since there were no values exceeding 10, we concluded that the model was free from multicollinearity.

Thus, we have the following equation for the dependent variable Mn:

$$Mn = -22.534 + 0.509Fe - 0.202Zn - 0.167Cu - 4.439Co + 15.823Cd - 0.018Pb \quad (1)$$

According to (1), a direct influence, increasing the resultant factor Mn, was found out for the factors Fe and Cd. Classification ranges given by model coefficients are presented in Table 3. Calculations were performed with a probability of 90% and 95%, respectively. Fe and Zn variables ranges were relevant in both cases of the evaluation.

Figure 1 graphically depicts the validity of regressors, i.e. variable plots and plots of residuals *versus* each regressor.

Figure 2 graphically depicts the differences in the calculation of actual data *vs*. predicted data from the model, which indicate the ability to perform some calculations for different values of the variable Mn of the factors included in the presented model.

CONCLUSIONS

Results of the studied area showed that the selected elements concentrations were below the safe limits of WHO/FAO set for edible mushrooms and for foodstuffs. This could be attributed to the lack of anthropogenic input like mining and industry and low-scale agricultural activities. From the obtained concentrations of heavy metals it could be concluded that the locality Batak Mountain, Bulgaria is an ecologically clean area, very suitable for collecting wild edible mushrooms for daily rations. Based on the obtained ordinary least squares linear regression model, the following interpretations for Mn contents in the wild edible mushroom *Suillus luteus* growing in the Batak Mountain, Bulgaria could be made:

- If Fe and Cd increase by 1%, the effect of this increase would result in an increase in Mn by 8.17% on the average;
- If Zn, Cu, Co and Pb decrease by 1%, the effect of this decrease would result in a decrease in Mn by 1.21% on the average.

REFERENCES

1. G. C. Wakchaure, in: Mushrooms Cultivation, Marketing and Consumption, Yugantar Prakashan Pvt. Ltd, New Delhi, 2011, p. 15.
2. N. Proskura, J. Podlasińska, L. Skopicz-Radkiewicz, *Chemosphere*, **168**, 106 (2017).
3. SWAiD. Central Statistical Office of Poland. Forest Use - Procurement of Forest Products, 2015.
4. E. Boa, Wild Edible Fungi, A Global Overview of Their Use and Importance to People, FAO, Rome, 2004.
5. P. Kalač, *Food Chem*., **122**, 2 (2010).
6. A. Frankowska, J. Zi´ołkowska, L. Bielawski, J. Falandysz, *Food Addit. Contam. Part B*, **3**, 1 (2010).
7. M. Aloupi, G. Koutrotsios, M. Koulousaris, N. Kalogeropoulos, *Ecotoxicol. Environ. Saf.*, **78**, 184 (2011).
8. P. Kalač, Edible mushrooms, chemical composition and nutritional value, Academic Press, London, 2016.
9. M. Ivanova, L. Dospatliev, P. Papazov, *C. R. Acad. Bulg.*, **72**, 182 (2019).
10. C. L. Keen, J. Y. Uriu-Adams, J. L. Ensuma, M. E. Gershwin, Handbook of nutrition and immunity, NJ: Humana Press, Totowa, 2004.
11. S. Sumaira, M. Ghulam, M. Hira, M. W. Connie, J. Yasir, S. Muhammad, *J. Food Nutr Res.*, **4**, 703 (2016).
12. S. Marek, P. Rzymski, P. Niedzielski, A. Budka, M. Gaşecka, P. Kalač, A. Jasińska, S. Budzyńska, L. Kozak, M. Mleczek, *Eur. Food Res. Technol.*, **243**, 1555 (2017).

13. M. Mleczek, P. Niedzielski, P. Kalač, A. Budka, M. Siwulski, M. Gaşecka, P. Rzymski, Z. Magdziak, K. Sobieralski, *Environ. Sci. Pollut. Res.*, **23**, 16280 (2016).
14. L. Dospatliev, M. Ivanova, *Oxid. Commun.*, **40**, 993 (2017).
15. L. Dospatliev, M. Ivanova, M. Lacheva, T. Radoukova, *Bulg. Chem. Commun.*, **50**, 538 (2018).
16. P. K. Ouzouni, D. Petridis, W. D. Koller, K. A. Riganakos, *Food Chem.*, **115**, 1575 (2009).
17. S. R. Koyyalamudi, S. C. Jeong, S. Manavalan, B. Vysetti, G. Pang, *J. Food Compost. Anal.*, **31**, 109 (2013).
18. L. Dospatliev, M. Ivanova, *Bulg. Chem. Commun.*, **49**, 5 (2017).
19. X. Wang, H. Liu, J. Zhang, T. Li, Y. Wang, *J. Environ. Sci. Health B*, **52**, 178 (2017).
20. S. S. Shapiro, M. B. Wilk, *Biometrika*, **52**, 591 (1965).
21. D. Gujarati, Basic Econometrics, McGraw-Hill, Singapore, 2003.
22. https://www3.nd.edu/˜wevans1/econ30331/Durbin_Watson_tables.pdf.
23. https://www.statisticssolutions.com/wp-content/uploads/wp-post-to-pdf-enhanced-cache/1/assumptions-of-multiple-linear-regression.pdf.
24. U. Udochukwu, B. Nekpen, O. Udinyiwe, F. Omeje, *IJCMAS*, **3**, 52 (2014).
25. J. Brzezicha-Cirocka, M. Mędyk, J. Falandysz, P. Szefer, *Environ. Sci. Pollut. R.*, **23**, 21517 (2016).
26. M. Gebrelibanos, N. Megersa, A. Taddesse, *Int. J. Food Contam.*, **3**, 1 (2016).
27. L. Dospatliev, M. Ivanova, *Chemistry*, **26**, 377 (2017).
28. J. Falandysz, A. Sapkota, A. Dryżałowska, M. Mędyk, X. Feng, *Environ. Sci. Pollut. R.*, **24**, 15528 (2017).
29. P. Rzymski, P. Klimaszyk, *Compr. Rev. Food Sci. Food Saf.*, 17(**5**), 1309 (2018).
30. C. Elekes, G. Busuioc, G. Ionita, *Not. Bot. Horti Agrobot. Cluj. Napoca*, **38**, 147 (2010).
31. R. Naresh, B. Udaya, R. Byragi, *IJES*, **3**, 28 (2012).
32. P. Kalač, *J. Sci. Food Agric.*, **93**, 209 (2013).
33. I. Okwulehie, J. Ogoke, *IJAMBR*, **1**, 7 (2013).
34. A. Quarcoo, G. Adotey, *Mycosphere*, **4**, 960 (2013).
35. P. George, T. Ranatunga, S. Reddy, G. Sharma, *Am. J. Food Technol.*, **9**, 360 (2014).
36. WHO. World Health Organization Evaluation of Certain Foods Additives and Contaminants (Twenty-Sixth Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Library Cataloguing-in-Publication Data, WHO: Geneva, Switzerland, 1982.
37. http://www.stat.purdue.edu/˜jtroisi/STAT350Spring2015/tables/FTable.pdf.